

Evaluation of a new generation of culture bottle using an automated bacterial culture system for detecting nine common contaminating organisms found in platelet components

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BACKGROUND: An automated bacterial culture system (BacT/ALERT 3D, bioMérieux) has been previously validated with a variety of bacteria in platelets. The recovery of bacteria in platelets using a new generation of culture bottles that do not require venting and that use a liquid emulsion sensor was studied.

STUDY DESIGN AND METHODS: *Bacillus cereus*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Serratia marcescens*, *Streptococcus viridans*, and *Propionibacterium acnes* isolates were inoculated into Day 2 platelets to concentrations of 10 and 100 CFU per mL. Samples were then studied with current and new aerobic, anaerobic, and pediatric bottles.

RESULTS: All organisms, except *P. acnes*, were detected in a mean time of 9.2 to 20.4 (10 CFU/mL) or 8.7 to 18.6 (100 CFU/mL) hours. *P. acnes* was detected in a mean time of 69.2 (10 CFU/mL) or 66.0 (100 CFU/mL) hours. The 10-fold increase in inoculum was associated with a mean 9.2 percent difference in detection time. The aerobic, anaerobic, and pediatric bottles had a mean difference in detection time (hours) between the current and new bottles of 0.10 ($p = 0.61$), 0.4 ($p = 0.38$), and 1.0 ($p < 0.001$), respectively.

CONCLUSION: No difference in detection time between the current and new aerobic and anaerobic bottles was demonstrated. The new pediatric bottles had a small but significant delay in detection.

Approximately 1 in 2000 platelet components are bacterially contaminated, and it is estimated that severe morbidity or death occurs in as many as 100 to 150 patients every year in the United States alone due to bacterially contaminated platelets.¹⁻³ The use of automated bacterial culture systems has been advocated as a means to minimize transfusion-transmitted sepsis and possibly extend platelet shelf life. We have previously validated one of them (BacT/ALERT 3D Microbial Detection System, bioMérieux, Durham, NC) with a variety of bacterial contaminants in platelets.⁴ Recently, the culture bottles used with this system were reconfigured to eliminate the need for venting of the aerobic bottles (bottles now have a higher pO_2 and an increased head space), and a colorimetric liquid emulsion sensor was substituted for the solid-state colorimetric sensor disk to simplify the production of the bottles. We compared the current generation of culture bottles to the new generation of culture bottles with nine seeded organisms in single-donor apheresis platelets.

MATERIALS AND METHODS

This study was approved by the University of North Carolina Committee on the Protection of the Rights of Human

ABBREVIATION: ATCC = American Type Culture Collection.

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TABLE 1. Inoculating concentrations of organisms

Organisms	Source	10 CFU/mL		100 CFU/mL	
		Inoculated (CFU/mL)	Recovered (CFU/mL)	Inoculated (CFU/mL)	Recovered (CFU/mL)
<i>Bacillus cereus</i>	ATCC 11778	4	<2	44	40
<i>E. coli</i>	ATCC 25922	17	8	169	190
<i>E. cloacae</i>	Clinical isolate	26	16	256	320
<i>K. oxytoca</i>	Clinical isolate	17	12	166	155
<i>P. acnes</i>	Clinical isolate	43	15	431	540
<i>S. aureus</i>	ATCC 27217	12	13	115	155
<i>S. epidermidis</i>	ATCC 49134	10	11	98	110
<i>S. marcescens</i>	ATCC 43862	19	<2	185	30
<i>Streptococcus viridans</i>	Clinical isolate	11	5	109	185
Mean*		18	9	175	192

* For the calculation of the mean, values below detection were taken at the limit of detection (e.g., <2 was taken as 2 CFU/mL), and all numbers were rounded to the nearest whole number. Mean bag volume \pm SD was 237 \pm 31 mL (range 187–281 mL).

Subjects (Durham, NC). The University of North Carolina Platelet Donor Program collected the apheresis platelets. All collections were from volunteer donors, and the apheresis platelets were purchased from the University of North Carolina Transfusion Medicine Service. All collections were performed with cell separators (Spectra or Trima, Gambro BCT, Lakewood, CO) and were WBC reduced as part of the collection process.

American Type Culture Collection (ATCC) or clinical isolates of nine microorganisms (see Table 1) were inoculated into Day 2 (day of collection equals Day 0) single-donor apheresis platelets to achieve both 10 and 100 CFU per mL in the platelet bag (2 platelet units/organism). Representative isolates of *Bacillus* sp., *Enterobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Serratia marcescens*, and *Streptococcus* sp. were chosen because these organisms accounted for 85 percent of the septic transfusion fatalities from platelets reported to the United States FDA from 1976 to 1998.⁵ *P. acnes*, which is of questionable clinical significance in platelets, was studied because of the prolonged lag phase observed in previous experiments.⁴ In the case of the *Enterobacteriaceae* family (*Klebsiella oxytoca*, *E. coli*, *S. marcescens*, and *Enterobacter cloacae*), isolates were selected plasma-resistant strains.

The inoculum of 10 CFU per mL was chosen because the few studies that have attempted to quantitate the degree of bacterial contamination on the day that the contamination was suspected have deduced that the majority of contaminated platelet components had bacterial contamination of at most 10 CFU per mL and because blood inoculation experiments with final inoculation concentrations of less than 5 CFU per mL are often complicated by inconsistent growth.^{6–14} In addition, it would be anticipated that in the vast majority of contaminated platelets the concentration of bacteria on Day 2 of storage would be greater than 10 CFU per mL.¹⁵

All units were inoculated with bacterial suspensions

obtained from several colonies grown on 5-percent sheep blood agar plates and incubated overnight. The turbidity of the suspensions was adjusted to match a 0.5 McFarland standard. Serial dilutions of these suspensions yielded concentrations from which small aliquots (0.5–5 mL) were inoculated into the platelet bags. The actual inoculum was confirmed from quantitative culture of the McFarland dilutions and with aliquots of the postinoculation platelets cultured on 5-percent sheep blood agar plates. To ensure a final bag concentration of 10 CFU per mL, both 10- and 100-CFU-per-mL bags were prepared (to allow for the vagaries of concentration that can be seen with nephelometry and McFarland standards).

Each bag was sampled five times (20 mL/sample with a syringe). Before bacterial inoculation, two samples from each bag were inoculated into both current- and new-generation standard aerobic and standard anaerobic bottles (4 mL each), and 2 mL of each sample was inoculated into current- and new-generation pediatric aerobic bottles (so as to maintain a 1 in 10 dilution) to ensure baseline sterility of the original apheresis bags. Additionally, three samples were obtained after the inoculation of the bags with bacteria and similarly placed into culture bottle sets. The new generation of pediatric bottles contain activated charcoal and are designed to minimize the effect of any antibiotics in a blood sample.

Thus, of the BacT/ALERT 3D bottles, 216 (108 current-generation bottles and 108 new-generation bottles) were inoculated with samples of sterile apheresis platelets, and 540 were inoculated with samples of apheresis platelets obtained after the inoculation with bacteria in these experiments.

All sampling and inoculum injections of the apheresis bags were performed aseptically through a sampling-site coupler. Quantitative cultures of the McFarland suspensions were performed by serial 1 in 10 dilutions of 0.1 mL of platelets on sheep blood agar and incubated at 35°C for 24 to 48 hours. Colonies were then counted from

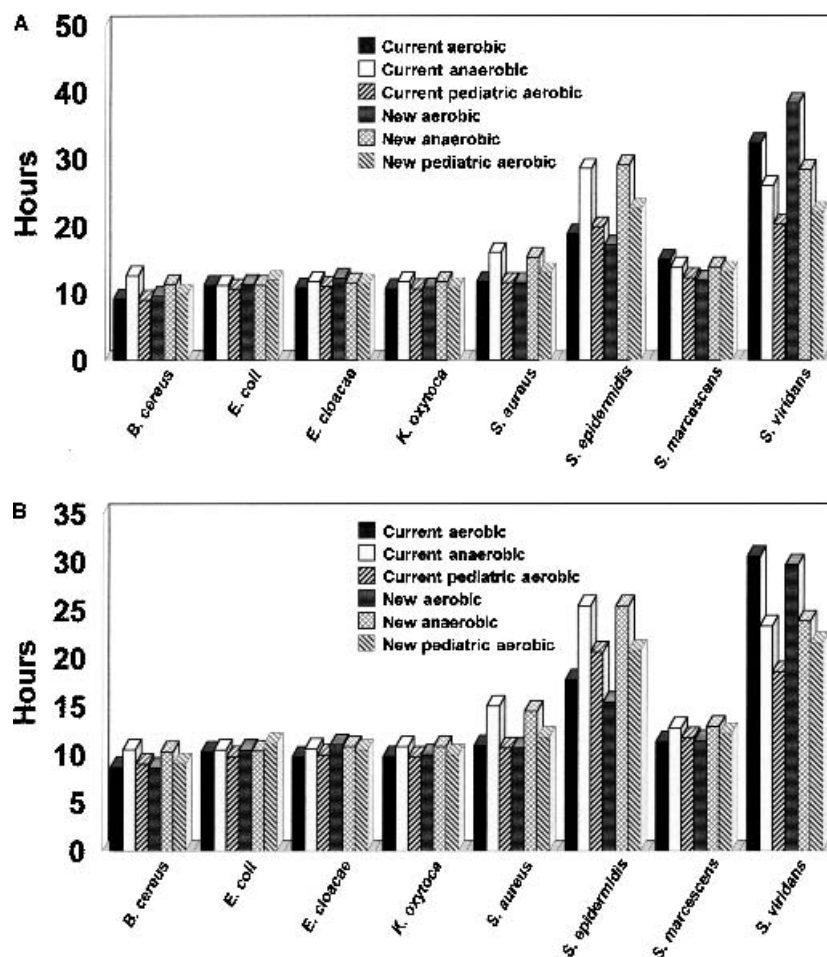


Fig. 1. (A) Mean time to detection displayed by bottle type for eight microbial organisms (excluding *P. acnes*) inoculated at 10 CFU per mL. Mean time to detection in inocula of 10 CFU per mL for all organisms with the exception of *P. acnes* ranged from 9.2 to 20.4 hours (all bottles). Target, 10 CFU per mL inoculum; actual mean, 18 CFU per mL. (B) Mean detection times for 100 CFU per mL inoculum for all organisms with the exception of *P. acnes* ranged from 8.7 to 18.6 hours (all bottles). Target, 100 CFU per mL inoculum; actual mean, 175 CFU per mL.

plates with 25 to 300 colonies, and the CFUs per mL were calculated. Postinoculation quantitative cultures were similarly performed by placing 0.1 mL (for the 100 CFU/mL inoculations) or 0.5 mL (for the 10 CFU/mL inoculations) of the platelet suspension on sheep blood agar and incubated at 35°C for 24 to 48 hours. The limit of culture sensitivity was 2 or 10 CFU per mL (depending on the volume plated). All cultures were performed in duplicate and the counts averaged.

Bottles were incubated in the BacT/ALERT 3D system until the system indicated that they were positive or until they had been incubated for 2 weeks. All positive bottles were Gram stained to confirm expected Gram-staining characteristics and morphology.

Statistical analysis

Comparisons between the current- and the new-generation culture bottles were performed using a Wil-

coxon's signed-rank test for paired data. Because the growth of *P. acnes* was inconsistent, delayed (if present) and unexpected in the standard aerobic and pediatric bottles, we were only able to analyze the standard anaerobic bottles in a paired analysis.

RESULTS

Final inoculum concentrations as determined by quantitative culture of McFarland dilutions and postinoculum aliquots are summarized in Table 1.

Times to detection (mean hours) of each organism by the BacT/ALERT 3D system are presented in Fig. 1 and Table 2. The average actual inoculum for 10 and 100 CFU per mL was 18 and 175 CFU per mL, respectively.

All organisms, with the exception of *P. acnes*, were detected in a mean time of 9.2 to 20.4 (10 CFU/mL) or 8.7 to 18.6 (100 CFU/mL) hours. *P. acnes* (Table 3) was de-

TABLE 2. Comparison of the mean \pm SD times to detection by inoculum concentration and bottle type*

	New-generation bottle (mean \pm SD)	Current-generation bottle (mean \pm SD)	Mean difference	Mean difference SD	p value
10 CFU/mL	14.5 \pm 4.8	13.3 \pm 4.2	1.2	0.9	<0.001
Pediatric aerobic	14.5 \pm 4.8	13.3 \pm 4.2	1.2	0.9	<0.001
Standard aerobic	15.5 \pm 9.1	15.1 \pm 7.7	0.4	3.8	0.333
Standard anaerobic	22.7 \pm 18.5	22.3 \pm 17.6	0.4	1.6	0.739
100 CFU/mL	13.4 \pm 4.7	12.6 \pm 4.4	0.9	1.3	0.001
Pediatric aerobic	13.4 \pm 4.7	12.6 \pm 4.4	0.9	1.3	0.001
Standard aerobic	13.5 \pm 6.7	13.7 \pm 7.2	0.2	1.2	0.777
Standard anaerobic	20.8 \pm 17.9	20.4 \pm 16.7	0.5	1.9	0.364
Total	14.0 \pm 4.8	12.9 \pm 4.2	1.0	1.1	<0.001
Pediatric aerobic	14.0 \pm 4.8	12.9 \pm 4.2	1.0	1.1	<0.001
Standard aerobic	14.5 \pm 8.0	14.4 \pm 7.4	0.1	2.8	0.605
Standard anaerobic	21.8 \pm 18.1	21.3 \pm 17.0	0.4	1.7	0.383

* The large SDs observed are due to the differences between times for different organisms. This is particularly true for the standard anaerobic bottles due to the inclusion of the slow growing *P. acnes*. SDs for each single organism with the exception of *P. acnes* were all less than 6.2 hours and most were less than 2 hours. Overall, there were no statistical differences between the pediatric and the standard aerobic new-generation bottles ($p = 0.484, 0.841, 0.056$ for 10, 100, and total CFU-mL, respectively).

TABLE 3. Comparison of the mean \pm SD times to detect *P. acnes* by inoculum concentration in standard anaerobic bottle

Inoculum	New bottle (mean \pm SD)	Current bottle (mean \pm SD)
10 CFU/mL	70.4 \pm 1.4	68.0 \pm 1.4
100 CFU/mL	68.0 \pm 2.8	64.0 \pm 1.4
Total	69.2 \pm 2.4	66.0 \pm 2.5

tected with the anaerobic bottles in a mean time of 69.2 (10 CFU/mL) or 66.0 (100 CFU/mL) hours. The difference in inoculating concentration (10 CFU/mL vs. 100 CFU/mL) was associated with an overall mean 9.2-percent difference in detection time. In aggregate (pooling organisms and concentrations), the aerobic bottles ($n = 48$ pairs) had a mean difference between current and new bottles of 0.10 hours ($p = 0.61$, Wilcoxon's signed-rank test), the anaerobic bottles ($n = 54$ pairs) had a mean difference of 0.4 hours ($p = 0.38$), and the pediatric bottles ($n = 48$ pairs) had a mean difference of 1.0 hours ($p < 0.001$).

In the 216 bottles (108 current-generation bottles and 108 new-generation bottles) that were inoculated with samples of sterile apheresis platelets, all remained nonreactive.

DISCUSSION

A variety of techniques have been suggested to screen platelets for bacterial contamination, but none have received general acceptance because lack of sensitivity, nonspecificity, excessive personnel time, expense, and a lack of a mandate (public or regulatory) to perform such testing.¹⁶ Currently, the most accepted method is the use of automated culture systems. Reports from Denmark, Yugoslavia, the Netherlands, and the United Kingdom

have advocated the use of bacterial culture of platelets on Days 2 or 3 to extend the shelf life of platelets to Day 7, thereby reducing the outdating of platelets and preserving a limited medical resource.¹⁷⁻²¹ Such a strategy is considered cost effective.^{17,21}

Validation of one such system with 13 aerobic bacteria known to contaminate platelets found that bacteria were detected in 9.2 to 25.6 hours when the starting concentration approximated 10 to 100 CFU per mL.⁴ Manufacturing changes made to the culture bottles required a revalidation in the context of platelet bacterial contamination to demonstrate comparability of the new bottles with the current bottles. This study found no difference between the new-generation and the current-generation aerobic or anaerobic bottles (at concentrations of 10 or 100 CFU/mL). However, the comparison of the new-generation and the current-generation pediatric aerobic bottles showed a small but significant difference in time to detection. This small difference was likely due to the inclusion of activated charcoal in the new pediatric bottles. The new generation of culture bottles was more efficient because of less handling time (a savings of 30-45 sec/bottle) and labor (venting was not performed) and were safer because of the elimination of the use of a needle in the venting step. Cost of the new-generation, standard aerobic and anaerobic bottles and the current bottles are comparable. The cost of the new pediatric bottles is higher because of the addition of activated charcoal.

As in the previous studies, growth of *P. acnes* required prolonged anaerobic culture time (60-70 hrs).⁴ However, *P. acnes*, which can occasionally be isolated from platelets, grows poorly in the aerobic environment of platelet storage and is of questionable clinical significance.

Growth studies of representative contaminants of platelets (*B. cereus*, *Pseudomonas aeruginosa*, *Klebsiella*

pneumoniae, *S. marcescens*, *S. aureus*, and *S. epidermidis*) have shown that cultures on Day 2 or 3 detect the vast majority of contaminated platelets.¹⁵ Cultures on Day 0 (day of collection) or Day 1 frequently have low concentrations of bacteria that may not be detected by culture.¹⁵ Implementation of Day 2 or 3 cultures (with a 2-day incubation time) could allow the extension of the shelf life of platelets.¹⁷⁻²² A testing paradigm shift would be required if implementation of such a policy occurs. Components would be issued before the completion of testing, recalled if the culture was positive, and the shelf life possibly extended to Day 7 if the culture remained negative.

Other practical issues to be considered before implementation of routine platelet culturing include the volume to be sampled, the number and type of bottles, and the acceptable specificity. For routine detection of microorganisms from blood and other normally sterile body fluids, 5 to 10 mL are recommended per bottle (package insert). Four-milliliter platelet samples per standard bottle were chosen for this study after consultation with the manufacturer, so as to maintain a 1 in 10 dilution of sample to media within the bottle. Higher volumes would be expected to have increased sensitivity but would result in a smaller remaining dose of platelets. Two bottles offer the advantage of increased sensitivity and could provide rapid confirmation of a reactive bottle. The need for an anaerobic culture in the context of platelet bacterial contamination has been debated. However, rare clinical cases of bacterial sepsis (e.g., *Clostridium perfringens*) after transfusion have been reported with obligate anaerobes.²³ Of note, as seen in Fig. 1, many aerobic organisms will grow in an anaerobic bottle. Pediatric bottles allow the use of smaller volumes, but in theory would be expected to have decreased sensitivity at low inoculums. Therefore, in establishing a bacterial surveillance program for platelets, we would envision the use of a minimum of 4-mL samples from 2- or 3-day-old platelets inoculated into both standard aerobic and anaerobic bottles. Ideally, testing should result in minimal unnecessary discards (<1%). In this set of experiments, all bottles inoculated with sterile platelets remained nonreactive (0 of 216), and a recent clinical culture study with the use of the BacT/ALERT 3D system of 2679 single-donor platelets demonstrated a false-positive rate of 0.6 percent.²²

We conclude that the new generation of standard aerobic and anaerobic culture bottles are equivalent to the current generation of culture bottles. An automated bacterial culture system could be used in either a blood collection center or a transfusion service to screen platelet products for bacterial contamination. Such testing would be expected to save lives and might allow an extension of platelet storage.

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